Remarks

Claims 1-11 and 13-19 were previously pending in this application. Claims 1-6 and 14-17 are amended herein. Claim 18 and 19 are canceled herein. Claims 5-11 and 13-17 are withdrawn from consideration. With entry of this amendment claims 1-4 are under consideration.

I. Amendments

The specification is amended to correct typographical errors.

The claims are amended as indicated in the listing of the claims, which replaces all prior versions of the claims in this application. No new matter is added by way of the amendments to the claims. Support for the amendments to the claims is found throughout the specification, claims and drawings as originally filed.

Claim 1 is now directed to "A monoclonal cell line". The remaining claims are amended to be consistent with amended claim 1. Support for the amendment is found at least at page 12 lines 1-24, page 25 lines 24-26 and page 29 lines 22-24 (Example 1), page 36 lines 18-24 (Example 2), and page 37 lines 21-27 (Example 3). Applicant has demonstrated monoclonality of the cells by FACS analysis (see Figures 3a and 9 showing a single peak in the antigen profiles).

Claim 1 is also amended to make even more clear that the claimed cell line has all of the recited properties. The amendment finds support at least at page 29 line 27 to page 30 line 17, page 34 line 3 to page 35 line 31, and page 37 lines 23-27.

Claim 2 is amended to make even more clear that the claimed cells express none of the recited markers. The amendment finds support at least at page 14 lines 12-19.

II. Double Patenting

The Examiner provisionally rejects claims 1 to 4 under 35 U.S.C. 101 as being in conflict with claims of co-pending U.S. application 10/521,071. Applicant has duly noted the Examiner's remarks and provisional rejection. In the event that claims 1 to 4 are otherwise found allowable,

Applicant respectfully requests withdrawal of this rejection in accordance with MPEP 804 (I)(B)(2).

III. Rejection of claims 1-4 and 18 under 35 U.S.C. §112 2nd paragraph

Claim 18 is cancelled thereby rendering the rejection moot with respect to this claim. Claims 1 and 4 are amended to "A monoclonal cell line which has all of the [recited] properties" (claim 1) and "wherein cells of the cell line do not express any of the [recited] markers" (claim 2). The amendments fully address the Examiner's concerns. Withdrawal of the rejection under 35 U.S.C. §112 2nd paragraph is requested.

IV. Rejection of claims 1-4 and 18 under 35 U.S.C. §112 1st paragraph

The Examiner alleges that claims 1 to 4 do not meet the enablement requirements because certain biological deposits have not been made. The Examiner again requires that certain exemplified cells such as HuSH cells, be deposited with an international depositary or otherwise made available to the public. Applicant traverses.

The Examiner asserts that the biological materials, specifically HuSH cells, are essential to the invention. The Examiner is incorrect. The invention involves deriving the claimed cell lines from various sources including embryos, embryonic stem cells, and bone marrow (page 3 line 29 to page 4 line 7). HuSH cells are only one example of such a cell line produced by Applicant starting with human bone marrow. Applicant has demonstrated monoclonal embryo-derived cell lines such as RoSH cells (Example 1), monoclonal bone marrow-derived cell lines such as Ro(BM)SH, PoSH and HuSH cells (Example 3), and at least 5 monoclonal ES-derived cell lines (Example 2). As described throughout the specification (see e.g. page 30 lines 14-15, page 36 lines 21-24), numerous cell lines were established of which RoSH, Ro(BM)SH, PoSH and HuSH are only examples. As is clear from the specification, the cell line RoSH was deposited for illustrative purposes only:

> "This cell line is illustrative only of the mammalian hemangioblast cell lines which can be obtained by the method of the invention." [Page 30 lines 22-24]

Furthermore,

> "The RoSH, Ro(BM)SH, PoSH and HuSH cell lines established by the applicant provide a useful reference for the characterization of hemangioblast. Hemangioblast cell lines are useful in characterizing or identifying early molecular events and molecules or factors in lineage commitment, differentiation and tissue organization during vasculogenesis, angiogenesis and hematopoiesis." [Page 20 lines 29-35]

The specification has therefore made clear that none of the specific designated cell lines including HuSH are essential to the invention.

The Examiner asserts that the specification does not describe a repeatable process to obtain the biological materials (RoSH2, Ro(BM)SH, HuSH and PoSH) which the Examiner considers to be essential. As stated above, the specific cell lines obtained by Applicant are only illustrative and are not essential. Applicant again points out that claims 1-4 do not recite specific cell lines. Only claim 5 specifically recites RoSH2, which Applicant has deposited with ATCC as required. The generically defined cell lines of claims 1-4 are fully enabled without the biological deposits.

The Examiner asserts that a skilled person would require undue experimentation to make the claimed cell population. Applicant disagrees.

According to the Examiner, the specification (at page 29, lines 3-26) describes culturing the cells from "older embryos" as "complex, with many different cell types and derivation of RoSH lines required extensive subcloning." Applicant submits that the Examiner has taken these statements out of context. The specification at page 29 describes in detail the culturing of cells to make the claimed cells, from isolating the putative hemangioblasts from 3.5 dpc blastocysts and 5.5 dpc delayed blastocysts, culturing them on normal gelatinized tissue culture plates, identifying, disaggregating the outgrowths and plating them on fibroblast feeder plates. Applicant showed in Figure 1A the colonies of fibroblastic cells that were observed after 2 to 4 weeks. From this procedure, Applicant established two cell lines adapted to grow on gelatinized plates, and stated that lines derived from delayed 5.5 dpc blastocysts usually arose from one colony per blastocyst. Applicant then stated:

> "To determine the upper limit of embryonic development from which isolation of these putative hemangioblasts was technically convenient, E6.0 to

> E7.5 embryos were isolated and the egg cylinders were dissected out. The egg cylinders were cultured on gelatinized, embryonic fibroblast feeder plates. It was observed that if the embryo proper was dissected for culture, growth of the embryo was poor but this can be remedied by placing the extraembryonic tissues besides the embryo. Once the embryo attached to the tissue culture plate and began to proliferate, the extraembryonic tissues were removed. By this means, hemangioblast lines have been isolated with relative ease from embryos as old as E7.0. Cultures of older embryos tend to produce complex mixtures of cell types. The frequency of deriving RoSH cell lines from delayed blastocysts was about 1 in 30 and that from E6.0 and older embryos was higher at about 1 in 10. Using delayed blastocysts to derive RoSH cell lines has the advantage of simplicity in establishing monoclonal lines. In all instances when RoSH lines were derived from delayed blastocysts, these lines arose from a single colony. Cultures of older embryos were complex with many different cell types and derivation of RoSH lines required extensive subcloning."

It is clear that Applicant was merely trying to give guidance for using older embryos. The statement that "Cultures of older embryos were complex with many different cell types and derivation of RoSH lines required extensive subcloning" clearly does not mean that the claimed cells are not enabled. Actually, Applicant has provided an extremely detailed teaching.

The Examiner asserts that Applicant has not provided a "reliable screening test" for enabling the invention. Applicant disagrees.

The Examiner asserts that Applicant's description of cells having "fibroblastic morphology", being "ring-like", and "forming a meshwork of cord-like structures at high confluency on gelatin-coated plates" is imprecise and unreliable. Applicant disagrees with the Examiner's characterization of Applicant's description. The descriptive passages the Examiner referred to are merely indicators of what a skilled person should look for; they are by no means the complete teaching. The claimed cell line has four characteristics which define it, and assays for these characteristics are routine; e.g. see page 29 line 27 to page 30 line 17 for continuous culture, page 34 lines 10-29 for inducing tumor formation, page 34 line 30 to page 35 line 31 for determining differentiation potential *in vitro* and *in vivo*, and page 29 line 27 to page 30 line 17 for maintaining inhibition of differentiation.

Example 1 describes exhaustively, and with photographic guidance, how to obtain cultured colonies from blastocysts and embryos and obtain monoclonal cell lines. In particular, the description at page 12 lines 1-17 stresses monoclonality:

> "Applicant has described the isolation and establishment of hemangioblast progenitor cell lines from mammalian embryos, from mammalian embryonic stem cells and from mammalian bone marrow, which have the potential to differentiate into both hematopoietic and endothelial cells. The establishment of monoclonality in these cell lines is preferred to obtain cell lines that have this bi-potentiality. The procedures are described which the applicant has taken at several stages of isolation to ensure monoclonality.
>
> During isolation from mammalian embryos, single colonies were selected from primary blastocyst or embryo cultures whenever possible. During isolation from mammalian bone marrow, single colonies were selected from individual bone marrow clumps whenever possible. In other situations, single cells were plated in 96-well plates by limiting dilution. Once a line was established, care was taken to clone the line by plating single cells in methylcellulose-based media."

Applicant has tested for differentiation *in vitro* (page 26 lines 5-17), vascularization of teratomas (page 27 lines 3-9), and incorporation of the claimed cells into liver vasculature (page 27 lines 10-27). Applicant has isolated at least 19 cell lines with each line originating from a single embryo (page 30 lines 13-17). Applicant has demonstrated monoclonality of the cells by FACS analysis (see Figures 3a and 9 showing a single peak in the antigen profiles).

Applicant submits that the claims comply fully with 35 U.S.C. §112 1st paragraph.

V. Rejection of the claims under 35 U.S.C. §102(b)

(a) Claims 1 to 4 are novel over Hughes et al., in light of Rafii et al.

The Examiner rejects claims 1-4 and 18 under 35 U.S.C. §102(b) as lacking novelty over Hughes et al., 1988 in "Methods in Bone Biology" Chapman & Hall, Arnett et al., eds, pages 20-22 ('Hughes'), in light of Rafii et al., June 2003 Nature Medicine 9(6):702-712 ('Rafii'). Applicant traverses.

The claims are for "A monoclonal cell line" which has four recited properties. Hughes describes isolation of whole bone marrow. Although Hughes describes cell culture conditions, these are specifically adapted for *transient* culture and differentiation of *primary stromal cells and osteoblasts* which give rise to bone, not hematopoietic or angioblastic cells. The paragraph bridging pages 20 and 22 of Hughes states: "Although studies demonstrate evidence of at least limited self-renewal of the *osteoprogenitor* cells within these cultures, particularly in the presence of dexamethasone [26,119], repeated subculture results in the loss of *osteoblastic* potential and *thus experiments with these cells are normally done on passage 1 cells*" [emphasis added]. Furthermore, the protocol on page 21 at step 10 directs the reader to "maintain cultures for *21 days*". Hughes describes the differentiation outcome of *marrow stromal cells into osteoblasts* (page 20 second paragraph) and states "cells are plated at low density and kept in culture for periods of *2-4 weeks*."

The Examiner asserts "The mere fact that the bone marrow of Hughes et al (according to Rafii et al.) comprises cells called "EPCs" and "HSCs" and the instant cells are not so called fails to distinguish the instant cells from the bone marrow of Hughes et al." The Examiner is incorrect. EPCs and HSCs do not have the properties of the claimed cells since the claimed cells have not yet differentiated into EPCs and HSCs. As can be seen from Table 1 on page 704 of Rafii, EPCs and HSCs are $CD34^+$ and $Sca1^+$ whereas the cell line of claim 2 are defined as $CD34^-$ and $Sca1^-$. EPCs are also $VEGFR2^+$ (i.e. Flk-1^+) whereas the cell line of claim 2 are Flk-1^-.

Hughes and Rafii do not teach making the claimed cell lines. The claimed subject matter is materially different from Hughes in light of Rafii. Withdrawal of the rejection with respect to Hughes and Rafii is requested.

(b) Claims 1 and 3 are novel over Kraus et al. (WO 00/11139)

The Examiner rejects claims 1, 3 and 18 under 35 U.S.C. §102(b) as lacking novelty over Kraus et al., WO 00/11139 ('Kraus1'). Applicant traverses.

As an initial matter, Applicant disagrees with the Examiner's statement: "Cord blood, and indeed the entire human body, arises from a single cell (the fertilized egg) and, as such, can

be considered to be "cultured from a single clone" as required by claim 18". Applicant submits that no skilled person would construe "cells cultured from a single clone" to be the same as the entire human body; nor would he think that a "single clone" is the same as a fertized egg, nor that "arising from a single cell" is the same as "cultured from a single clone". Applicant has canceled claim 18 solely to advance prosecution and does not concede to the Examiner's argument.

With respect to the currently submitted claims, the claims are for "A monoclonal cell line" which has four recited properties. Kraus1 describes cell populations enriched in stem cells that can be expanded (page 2 lines 1-6), and methods for increasing the proportion of non-fetal hemangioblasts in the primary cell culture population (page 3 lines 28-34). Kraus1 describes starting the process with a population that contains at least 10% fewer non-fetal hemangioblasts than the enriched population (page 4 lines 32-34) to yield mixed cell populations containing at least 2%, and up to 25%, human non-fetal uncommitted hemangioblasts (page 4 lines 9-16). Kraus1 selected for the human non-fetal uncommitted hemangioblasts using negative or positive selection schemes, e.g. antibody-based affinity binding (page 7 line 4 to page 8 line 7). The Examiner does not point to, nor is Applicant aware of, any disclosure in Kraus1 that teaches selecting or culturing a single clone or any attempt, much less success, at creating a monoclonal cell line, as currently claimed.

Claim 2 is further distinguished from Kraus1's disclosure since the cell line of claim 2 does not express Flk-1. In contrast, Kraus1 characterized their uncommitted human hemangioblasts as being Flk-1^+ (page 4 lines 22-24, page 6 lines 20-23, and claim 18).

Kraus1 does not teach making the claimed cell lines. Applicant submits the claimed subject matter is materially different from Kraus1 and is novel over Kraus1.

Applicant further submits that the Examiner's stated position on written description and enablement of hemangioblasts contradicts the Examiner's arguments on inherent anticipation. Kraus1 describes hemangioblasts without characterizing them by the four properties recited in Applicant's claim 1. The Examiner doubts whether hemangioblasts exist (see Office Action dated Jun 27, 2005) yet now says that the four recited properties, though not disclosed, are inherent in Kraus1's cells. How can Applicant's claimed cells be

the same as Kraus1's "hemangioblasts", whose existence the Examiner doubts? According to the Office Action dated Jun 27, 2005:

> "Even if a single precursor for the hematopoietic and endothelial lineages does exist (which the examiner certainly does not concede), a person of ordinary skill in the art would not have a reasonable expectation that the cells instantly described and claimed by applicants are said precursor."

The Examiner doubts that the same progenitor cell can give rise to both hematopoietic and endothelial lineages (see Office Action dated Jun 27, 2005). Yet Applicant has described and shown the claimed cells' potential to differentiate into hematopoietic and endothelial lineages (see page 32 line 1 to page 35 line 31, and page 38 line 25 to page 39 line 4), and demonstrated that these cells are monoclonal (see Figures 3a and 9 showing a single peak in the antigen profiles).

The Examiner reasoned that a mixed cell population such as bone marrow contains progenitors that separately differentiate into hematopoietic and endothelial lineages, and that such a cell population therefore anticipates "A purified preparation of mammalian cells" (as recited in former claim 1). By the Examiner's own reasoning, Kraus1's cell population cannot be the same as the cell lines presently claimed by Applicant which are required to be monoclonal.

The claims are novel over Kraus1. Withdrawal of the rejection with respect to Kraus1 is requested.

VI. Rejection of the claims under 35 U.S.C. §102(e)

(a) Claims 1 and 4 are novel over Scott et al.

The Examiner rejects claims 1-4 and 18 under 35 U.S.C. §102(e) as lacking novelty over Scott et al., US application publication 2003/0180265 ('Scott'). Applicant traverses.

Scott describes a method a method for isolating an hemangioblast from the bone marrow of an adult animal. This method includes the steps of isolating bone marrow from the animal and separating hemangioblasts from non-hemangioblasts by methods such as antibody affinity purification [0008], [0042] and [0015]. Scott also describes a hemangioblast

isolated from adult bone marrow [0014], as well as methods for modulating angiogenesis in a subject by administering to the subject hemangioblasts (possibly expanded *in vitro*) derived from the subject [0010]. Specifically, Scott describes purifying a cell suspension from bone marrow, then selecting for hemangioblasts by antibody-based negative and positive selection schemes [0058]. Scott describes transplanting either about 1000 re-purified hemangioblasts or a single isolated hemangioblast [0058] [0062] [0065].

Scott does not describe culturing a single clone to establish a cell line. Scott simply does not describe any attempt to create a monoclonal cell line, as claimed.

Claim 2 is further distinguished from Scott's disclosure since the cell line of claim 2 does not express CD34 nor Sca-1. In contrast, Scott characterized their hemangioblasts as being positive for CD34 [0025] and for Sca-1 by selection [0058].

Scott does not teach making the claimed cell lines. Applicant submits the claimed subject matter is materially different from Scott and is novel over Scott.

Applicant further submits that the Examiner's stated position on written description and enablement of hemangioblasts contradicts the Examiner's arguments on inherent anticipation. Scott describes hemangioblasts without characterizing them by the four properties recited in Applicant's claim 1. The Examiner doubts whether hemangioblasts exist (see Office Action dated Jun 27, 2005) yet now says that the four recited properties, though not disclosed, are inherent in Scott's cells. How can Applicant's claimed cells be the same as Scott's "hemangioblasts", whose existence the Examiner doubts? According to the Office Action dated Jun 27, 2005:

> "Even if a single precursor for the hematopoietic and endothelial lineages does exist (which the examiner certainly does not concede), a person of ordinary skill in the art would not have a reasonable expectation that the cells instantly described and claimed by applicants are said precursor."

The Examiner doubts that the same progenitor cell can give rise to both hematopoietic and endothelial lineages (see Office Action dated Jun 27, 2005). Yet Applicant has described and shown the claimed cells' potential to differentiate into hematopoietic and endothelial lineages (see page 32 line 1 to page 35 line 31, and page 38 line 25 to page 39 line 4), and

demonstrated that these cells are monoclonal (see Figures 3a and 9 showing a single peak in the antigen profiles).

The Examiner reasoned that a mixed cell population such as bone marrow contains progenitors that separately differentiate into hematopoietic and endothelial lineages, and that such a cell population therefore anticipates "A purified preparation of mammalian cells" (as recited in unamended claim 1). By the Examiner's own reasoning, Scott's cell population cannot be the same as the cell lines presently claimed by Applicant which are required to be monoclonal.

The claims are novel over Scott. Withdrawal of the rejection with respect to Scott is requested.

(b) Claims 1 to 4 are novel over Furcht et al.

The Examiner rejects claims 1-4 and 18 under 35 U.S.C. §102(e) as lacking novelty over Furcht et al., US application publication 2004/0107453 ('Furcht'). Applicant traverses.

Furcht describes a method for isolating and propagating a multipotent adult stem cell (MASC) from mammalian tissue by establishing a population of adherent cells, depleting the population of CD45+ cells, recovering CD45- cells and culturing them to produce an expanded cell population [0029] [0033] [0036]. Furcht describes administering the MASCs to patients as a medical treatment [0031].

Specifically, Furcht describes a continuous culture of an original population of primary bone marrow mononuclear cells (BMMNC), muscle or brain suspension [0097]. There was no selection of individual clones for establishing a monoclonal cell line. Furcht selected for $CD45^-/GlyA^-$ cells and plated them at 10 cells per well [0097], 1% of which yielded continuous growing cultures [0100]. Furcht also selected for $CD45^-/GlyA^-$ cells from bone marrow and expanded them [0110] [0111]. Nowhere did Furch describe establishing or even attempting a monoclonal cell line.

Furcht demonstrated certain properties of the isolated MASCs. To prove single cell origin of mesodermal and neuroectodermal differentiated progeny, Furcht retrovirally transduced a

population of isolated MASCs, induced them to differentiate, and identified the retroviral integration site as coming from the same progenitor cells [0141] [0142]. To see if MASCs can differentiate *ex vivo* to functional neuron type cells, Furcht used a single isolated MASC [0280]. To determine optimal conditions for MASC differentiation into hepatocyte-like cells, Furcht again used a population of isolated MASCs [0246]. To treat hemophilic mice, Furcht used 10^6 isolated MASCs from a transgenic mouse for transplantation [0280]. Again, nowhere did Furch describe a monoclonal cell line for any of these uses.

Furcht does not teach making the claimed cell lines. Applicant submits the claimed subject matter is materially different from Furcht and is novel over Furcht. Withdrawal of the rejection with respect to Furcht is requested.

(c) Claims 1 and 3 are novel over Kraus et al. (US patent 6,429,012)

The Examiner rejects claims 1, 3 and 18 under 35 U.S.C. §102(e) as lacking novelty over Kraus et al. US patent 6,429,012 ('Kraus2'). Applicant traverses.

Kraus2 describes cell populations enriched in stem cells that can be expanded (Column 1 lines 48-51), and methods for increasing the proportion of non-fetal hemangioblasts in the primary cell culture population (Column 2 lines 36-41). Kraus2 describes starting the process with a population that contains at least 10% fewer non-fetal hemangioblasts than the enriched population (Column 3 lines 4-10) to yield mixed cell populations containing at least 2%, and up to 25%, human non-fetal uncommitted hemangioblasts (Column 3 lines 11-15). Kraus2 selected for the human non-fetal uncommitted hemangioblasts using negative or positive selection schemes, e.g. antibody-based affinity binding (Column 4 lines 13-48). Kraus2 does not describe selecting or culturing a single clone, nor did Kraus2 describe any attempt to create a monoclonal cell line, as claimed.

Claim 2 is further distinguished from Kraus2's disclosure since the cell line of claim 2 does not express Flk-1. In contrast, Kraus2 characterized their uncommitted human hemangioblasts as being Flk-1^+ (Column 3 lines 15-19 and lines 63-66).

Kraus2 does not teach making the claimed cell lines. Applicant submits the claimed subject matter is materially different from Kraus2 and is novel over Kraus2.

Applicant further submits that the Examiner's stated position on written description and enablement of hemangioblasts contradicts the Examiner's arguments on inherent anticipation. Kraus2 describes hemangioblasts without characterizing them by the four properties recited in Applicant's claim 1. The Examiner doubts whether hemangioblasts exist (see Office Action dated Jun 27, 2005) yet now says that the four recited properties, though not disclosed, are inherent in Kraus2's cells. How can Applicant's claimed cells be the same as Kraus2's "hemangioblasts", whose existence the Examiner doubts? According to the Office Action dated Jun 27, 2005:

> "Even if a single precursor for the hematopoietic and endothelial lineages does exist (which the examiner certainly does not concede), a person of ordinary skill in the art would not have a reasonable expectation that the cells instantly described and claimed by applicants are said precursor."

The Examiner doubts that the same progenitor cell can give rise to both hematopoietic and endothelial lineages (see Office Action dated Jun 27, 2005). Yet Applicant has described and shown the claimed cells' potential to differentiate into hematopoietic and endothelial lineages (see page 32 line 1 to page 35 line 31, and page 38 line 25 to page 39 line 4), and demonstrated that these cells are monoclonal (see Figures 3a and 9 showing a single peak in the antigen profiles).

The Examiner reasoned that a mixed cell population such as bone marrow contains progenitors that separately differentiate into hematopoietic and endothelial lineages, and that such a cell population therefore anticipates "A purified preparation of mammalian cells" (as recited in former claim 1). By the Examiner's own reasoning, Kraus2's cell population cannot be the same as the cell lines presently claimed by Applicant which are required to be monoclonal.

The claims are novel over Kraus2. Withdrawal of the rejection with respect to Kraus2 is requested.

VII. Rejection of the claims under 35 U.S.C. §102(a)

(a) Claims 1 and 3 are novel over Miyajima et al.

The Examiner rejects claims 1, 3 and 18 under 35 U.S.C. §102(a) as lacking novelty over Miyajima et al., 2002 EP 1229116 ('Miyajima'). Applicant traverses.

Applicant's invention date is earlier than the reference date. Miyajima was published on August 7, 2002. The present application validly claims priority to US 60/453,729 filed on July 12, 2002. Applicant believes no affidavit or declaration under 37 CFR 1.131 is necessary. MPEP Section 715 (II) states: "(D) Where the effective filing date of applicant's or patent owner's parent application or an International Convention proved filing date is prior to the effective date of the reference, an affidavit or declaration under 37 CFR 1.131 is unnecessary because the reference should not have been used."

Miyajima is not prior art against the present application. Withdrawal of the rejection is requested.

V. Concluding Remarks

In view of the above amendments and remarks, reconsideration and favorable action on all pending claims are respectfully requested. If any questions or issues remain, the Examiner is respectfully requested to contact the undersigned prior to preparation of any additional written action.

Respectfully submitted,

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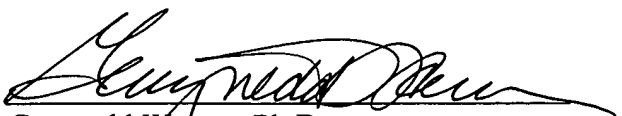
By 
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